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(71) Applicant: CISTRON BIOTECHNOLOGY, INC. [US/US]; 10 Bloomfield Avenue, Box 2004, Pine Brook, NJ 07058 (US).

(72) Inventors: DONDERO, Richard, S.; 37 Hillside Avenue, Riverdale, NJ 07457 (US). JANDINSKI, John; 8 Beverly Road, Madison, NJ 07940 (US).

(74) Agents: SALIWANCHIK, David, R. et al.; Saliwanchik, Lloyd & Saliwanchik, A Professional Association, Suite A–1, 2421 N.W. 41st Street, Gainesville, FL 32606–6669 (US).

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(54) Title: METHOD FOR INHIBITING CLEAVAGE OF PRECURSOR IL–1β

(57) Abstract

The subject invention concerns a method for inhibiting cleavage of precursor IL–1β in a human or animal. This process is useful to treat humans or animals hosting a medical condition wherein the presence of mature IL–1β is detrimental to the healing process of the afflicted human or animal.

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DESCRIPTION

METHOD FOR INHIBITING CLEAVAGE OF PRECURSOR IL-1β

Background of the Invention

Interleukin 1β (IL-1β) is a polypeptide hormone synthesized and secreted by stimulated monocytes. The initial translation product of IL-1β is a 31 kDa precursor polypeptide having relatively low biological activity. After synthesis, the 31 kDa precursor for IL-1β is enzymatically cleaved to its highly active mature form which has a size of about 17.5 kDa. The N-terminus of mature IL-1β derived from human activated monocytes has been characterized by an N-terminal amino acid sequence beginning with Ala-Pro. The N-terminal Ala residue of human mature IL-1β is in the 117 position and an Asp residue is in the 116 position counting from the N-terminus of human precursor IL-1β polypeptide. Mature IL-1β consists of the C-terminal 153 residues of the precursor polypeptide.

Many physiological actions and biological activities of IL-1β have been identified. IL-1β biological activity is often determined by assaying for stimulation of thymocyte proliferation. IL-1β activities include stimulation of B-lymphocyte maturation, lymphocyte proliferation, stimulation of fibroblast growth and induction of acute-phase protein synthesis by hepatocytes.

Other biological activities have been attributed to IL-1 polypeptides. These include control of differentiation and activation of limphocytes, stimulation of lymphokine and prostaglandin production, promotion of inflammation, induction of acute phase proteins, stimulation of bone resorption, and alteration of the level of iron and zinc in blood. Moreover, it has been found that IL-1 can stimulate the hypothalamus-pituitary-adrenal axis, suggesting that IL-1 is integrated in the complex neuroendocrine network that controls homeostasis.

Maturation and release of mature IL-1β from macrophages does not proceed by conventional means normally associated with most secretory proteins because the precursor IL-1β polypeptide lacks a hydrophobic signal sequence. Further, IL-1β is not associated with a membrane-bound compartment in monocytes. Most secretory proteins are characterized by the presence of a hydrophobic stretch of amino acids called a signal sequence. The signal sequence directs the translocation of the protein across the membrane of the endoplasmic reticulum during protein synthesis. The protein is subsequently ushered out of the cell via exocytosis. Most secreted proteins have a signal sequence at the amino terminal that is removed upon translocation. Other proteins, such as ovalbumin, have an internal signal sequence that is not removed upon translocation. The precursor form of IL-1β lacks any region (either amino

terminal or internal) with sufficient hydrophobicity and length to qualify as a signal sequence. Much remains to be learned about the synthesis, processing and secretion of IL-1β.

The discovery of a compound which specifically inhibits the cleavage of IL-1 will not only contribute to the understanding of how this molecule is synthesized, processed and secreted, but will also provide a therapeutic approach for diseases in which excessive or unregulated IL-1 production is implicated.

Though the mature form of IL-1β has been found to be beneficial in some medical conditions, there are medical conditions where its presence is not beneficial. Accordingly, where such a condition exists, it is desirable that mature IL-1β not be present, or, at least its level significantly reduced. The subject invention deals directly with this situation by providing a method for inhibiting cleavage of precursor IL-1β to mature IL-1β.

Brief Summary of the Invention

The subject invention provides materials and methods for inhibiting the production of mature interleukin-1β by monocytes and/or macrophages. In a specific embodiment, the method of the subject invention comprises administering to a human an effective amount of a tetracycline compound. It has been found that tetracycline compounds, including derivatives, analogs and salts of tetracycline, inhibit the cleavage of IL-1β to its most active mature form.

Upon treating a human having a condition wherein the presence of mature IL-1β is not desired, with an effective amount of tetracycline, or a tetracycline derivative, salt, or analog (hereinafter referred to collectively as tetracycline compounds), there results a decrease of mature IL-1β in said human. This decrease, which results from the action of tetracycline in inhibiting the cleavage of precursor IL-1β to mature IL-1β, provides therapeutic benefits as described herein.

Detailed Disclosure of the Invention

The subject invention provides materials and methods for reducing the production of active forms of polypeptide hormones. In a specific embodiment, the subject invention concerns the use of tetracycline, or derivatives, salts, or analogs thereof (tetracycline compounds), to inhibit the cleavage of precursor IL-1 to mature IL-1 in humans or animals. Although the presence of mature IL-1β is beneficial in some medical conditions, it also has been found to be detrimental in other situations. Where the medical condition is one not helped by the presence of mature IL-1β, then the subject invention can be used to reduce the presence of mature IL-1β and, thus, provide a therapeutic benefit.

In a preferred embodiment, the subject invention provides a method of inhibiting the cleavage of IL-1 by monocytes and/or macrophages in a human in need thereof which comprises administering an effective IL-1 cleavage inhibiting amount of a tetracycline compound. A tetracycline compound can be administered to such human in a conventional dosage form prepared by combining a tetracycline compound with a conventional pharmaceutically acceptable carrier or diluent according to known techniques.

As used herein, the term "inhibiting the cleavage of IL-1β" refers to a reduction in the production of the biologically highly active forms of IL-1β including the 17.5 kDa form which is frequently referred to as the "mature" form of IL-1β.

The tetracycline compound used according to the subject invention can be the parent form of tetracycline or any tetracycline derivative, analog, or salt which retains the ability to inhibit the cleavage of precursor IL-1β to a mature form. One tetracycline derivative which can be used according to the subject invention is doxycycline. Other antibiotics which act utilizing the same, or analogous, modes of action can be used so long as these compounds inhibit the production of the mature form of IL-1β. Without being limited by theory or constrained to a specific mechanism, one embodiment of the subject invention involves the inhibition of IL-1β cleavage by interfering with the enzymatic activity of IL-1β converting enzyme (ICE). The interference with ICE may result from a reduction in the expression of ICE, an interruption in the enzymatic activation of ICE, and/or interference with the ability of ICE to associate with and cleave IL-1β. Although the invention is exemplified herein with reference to tetracycline, its derivatives, salts, and analogs (tetracycline compounds) it should be understood that these teachings are applicable, and include within their scope, the use of other antibiotic compounds which possess the IL-1β cleavage inhibiting properties described herein. Specifically, the property of greatest interest is the inhibition of the cleavage of precursor IL-1β to mature IL-1β. In a preferred embodiment, this inhibition of cleavage occurs intracellularly such that, for example, in a cell culture of stimulated monocytes treated with the inhibitor there is observed a relative decrease, within the cells, of mature IL-1β and a relative increase of precursor IL-1β. Typically, such a relative change in the abundance of IL-1β species can be observed when the inhibitor is applied at concentrations of about 5 μg/ml and more and at a time period of about 24 to 72 hours or more.

When using the tetracycline compounds according to the subject invention it is preferable to use tetracycline compounds having a minimal antibiotic property and a maximum precursor IL-1β cleavage inhibiting property. Determination of these properties can be readily

ascertained by a person skilled in the art having the benefit of the instant disclosure and using well-known and available procedures.

Medical conditions which are presently known to be adversely affected by the presence of mature IL-1β include arthritis, inflammation, periodontal disease, and degenerative bone diseases. This should not be interpreted as an exhaustive list. It is merely exemplary of disease where the presence of mature IL-1β is not beneficial to the affected subject.

Interleukin-1 (IL-1) has been demonstrated to mediate a variety of biological activities thought to be important in immunoregulation and other physiological conditions such as inflammation [See, e.g., Dinarello *et al.*, *Rev. Infect. Disease*, 6:51 (1984)]. The myriad of known biological activities of IL-1 include the activation of T helper cells, induction of fever, stimulation of prostaglandin or collagenase production, neutrophil chemotaxis, induction of acute phase proteins and the suppression of plasma iron levels. There are several disease states in which excessive or unregulated IL-1 production by monocytes and/or macrophages is implicated in exacerbating and/or causing the disease. These include rheumatoid arthritis [See, e.g., Fontana *et al.*, *Arthritis Rheum.* 22:49-53 (1982)]; osteoarthritis [See e.g., Wood *et al.*, *Arthritis Rheum.* 26:975 (1983)]; toxic shock syndrome [See, e.g., Ikejima and Dinarello, *J. Leukocyte Biology* 37:714 (1985)]; other acute or chronic inflammatory disease states such as the inflammatory reaction induced by endotoxin [See, e.g., Habicht and Beck, *J. Leukocyte Biology* 37:709 (1985)]; and other chronic inflammatory disease states such as tuberculosis. [See, e.g., Chesque *et al.*, *J. Leukocyte Biology* 37:690 (1985)]. Benjamin *et al.*, "Annual Reports in Medicinal Chemistry-20", Chapter 18, pages 173-183 (1985), Academic Press, Inc., disclose that excessive IL-1 production is implicated in: Psoriatic arthritis, Reiter's syndrome, Rheumatoid arthritis, Osteoarthritis, Gout, Traumatic arthritis, Rubella arthritis, and Acute synovitis.

Dinarello, J. Clinical Immunology 5(5):287-297 (1985), reviews the biological activities which have been attributed to IL-1 and such activities are summarized in Table A.

Table A. Biological Activities Attributed to IL-1
Fever
Hypoferremia
Hypozincemia
Hypercupremia
Increased:
Blood neutrophils
Hepatic acute-phase proteins
Bone resorption
Cartilage breakdown
Muscle proteolysis
Slow-wave sleep
Endothelial procoagulant
Chondrocyte proteases
Synovial colagenase
Endothelial neutrophil adherence
Neutrophil degranulation
Neutrophil superoxide
Interferon production
Proliferation of
Fibroblasts
Glial cells
Mesangial cells
Synovial fibroblasts
EBV B-cell lines
Chemotaxis of
Monocytes
Neutrophils
Lymphocytes
Stimulation of PGE_2 in
Hypothalamus
Cortex
Skeletal muscle
Dermal fibroblast
Chondrocyte
Macrophage/monocyte
Endothelium (PGI_2)
Decreased
Hepatic albumin synthesis
Appetite
Brain binding of opioids
Augmentation of
T-cell responses
B-cell responses
NK activity
IL-2 production
Lymphokine production

In accordance with the subject invention, an effective IL-1 cleavage inhibiting amount of a tetracycline compound is useful in treating, prophylactically or therapeutically, any disease

state in a human which is exacerbated or caused by excessive or unregulated mature IL-1 production by such human's monocytes and/or macrophages.

The compounds useful according to the subject invention can be administered to a human or animal using methods well known in the art. The amount and concentration of compound used should be within the well-known toxicity level of these compounds, and an amount which maintains or reduces the level of mature IL-1β. This level can be monitored readily by use of standard well-known tests for mature IL-1β. It is best to administer the tetracycline compound at the low end of known dosage forms and then test the biological fluid for mature IL-1β. Once there is shown to be a desired level of mature IL-1β in the biological fluid, then the tetracycline compound dosage can be maintained at that level until suitable therapeutic effects are evidenced in the human or animal subject. It is well known in the art that subjects of different weights and/or proportions will require different levels of drug dosage to achieve the desired result. The means and knowledge for adjusting the dosage of the tetracycline compounds are well-known and available to those practicing in the art having the benefit of the instant disclosure.

It will be recognized by one of skill in the art having the benefit of the instant disclosure that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount and nature of active ingredient with which it is to be combined, the route of administration and other well-known variables. A tetracycline compound is administered to a human in need of inhibition of mature IL-1 production by its monocytes and/or macrophages in an amount sufficient to inhibit cleavage of precursor IL-1. The route of administration may be oral, parenteral or topical. The term parenteral as used herein includes intravenous, intramuscular, subcutaneous intranasal, intrarectal, intravaginal or intraperitoneal administration. The subcutaneous and intramuscular forms of parenteral administration are generally preferred. The daily oral dosage regimen will preferably be from about 5 to about 100 mg/kilogram of total body weight. The daily parenteral dosage regimen will preferably be from about 2 to about 80 mg per kilogram (kg) of total body weight, most preferably from about 3 to about 60 mg/kg. The daily topical dosage regimen will preferably be from about 2 mg to about 10 mg per site of administration. It will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of a tetracycline compound will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular patient being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses of a tetracycline compound given per day for a defined

number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

Accordingly, the process of the subject invention can be used to treat any human or animal afflicted with a medical condition in which the presence of mature IL-1β is not beneficial to the subject. As disclosed above, the compounds can be administered to a human or animal host via routes known for these compounds.

It should also be noted that the teachings of the subject invention can be used to improve prophylactic and therapeutic treatments wherein the presence of mature IL-1β is desired. Thus, for example, when would healing is needed or promoted, or when administering a vaccine, these procedures should be performed in the absence of a tetracycline compound.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

Claims

1. A method for inhibiting the cleavage of precursor IL-1β to mature IL-1β in a human which comprises administering to said human an effective precursor IL-1β cleavage inhibiting amount of a tetracycline compound.

2. The method, according to claim 1, wherein said tetracycline compound is administered to said human parenterally.

3. The method, according to claim 1, wherein said tetracycline compound is administered to said human intravenously or intramuscularly.

4. The method, according to claim 1, wherein said tetracycline compound is administered to said human orally.

5. The method, according to claim 1, wherein said inhibition of cleavage of IL-1β is used to treat a condition selected from the group consisting or arthritis, inflammation, periodontal disease, and degenerative bone disease.

6. A process for treating a human having excess mature IL-1β which comprises administering to said human an effective precursor IL-1β cleavage inhibiting amount of a tetracycline compound.

7. The process, according to claim 6, wherein said inhibition of cleavage of precursor IL-1β is used to treat a condition selected from the group consisting of arthritis, inflammation, periodontal disease, and degenerative bone disease.

8. The process, according to claim 6, wherein said tetracycline compound is administered to said human by a means selected from the group consisting of parental, intravenous, intramuscular and oral.

9. A process for treating a human or animal having a medical condition wherein the presence of mature IL-1β in said human or animal is detrimental wherein said method comprises administering to said human or animal an effective precursor IL-1β cleavage inhibiting amount

of a tetracycline compound wherein said tetracycline compound is characterized by having little or no antibiotic activity.

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(71) Applicant: CISTRON BIOTECHNOLOGY, INC. [US/US]; 10 Bloomfield Avenue, Box 2004, Pine Brook, NJ 07058 (US).

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(74) Agents: SALIWANCHIK, David, R. et al.; Saliwanchik, Lloyd & Saliwanchik, A Professional Association, Suite A-1, 2421 N.W. 41st Street, Gainesville, FL 32606-6669 (US).

(81) Designated States: AU, CA, IL, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT. LU, MC, NL, PT, SE).

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30 December 1998 (30.12.98)

(54) Title: INHIBITION OF THE CLEAVAGE OF PRECURSOR IL-1β

(57) Abstract

The subject invention concerns a method for inhibiting cleavage of precursor IL-1β in a human or animal. This process is useful to treat humans or animals hosting a medical condition wherein the presence of mature IL-1β is detrimental to the healing process of the afflicted human or animal.

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INTERNATIONAL SEARCH REPORT

Ir. ional Application No
PCT/US 98/05670

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/65

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 701 320 A (HASEGAWA KENJI ET AL) 20 October 1987 see column 2, line 24 - line 30 ---	1,5-7
X	LAMSTER ET AL: "the effect of tetracycline fiber therapy on beta-glucuronidase and interleukin-1 beta in crevicular fluid" J CLIN PERIODONTOL, vol. 23, no. 9, 1996, pages 816-822, XP002081052 see abstract --- -/--	1,5-7

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 October 1998	27/10/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040. Tx. 31 651 epo nl. Fax: (+31-70) 340-3016	Trifilieff-Riolo, S

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EADY ET AL: "modulation of comedonal levels of IL-1 in acne patients treated with tetracyclines" J OF INVESTIGATIVE DERMATOLOGY, vol. 101, no. 1, 1993, pages 86-91, XP002081053 see page 90; table V see page 90, right-hand column, paragraph 2 - paragraph 3 ---	1,5-7,9
X,P	BALLINGER ET AL: "tetracycline inhibits pro-inflammatory cytokine release from mononuclear cells..." GASTROENTEROLOGY, vol. 112, no. 4, April 1997, page 926 XP002081054 see abstract ---	1,2,5-9
Y	ARSENIS ET AL: "tetracyclines inhibit the synthesis and/or activity of cartilage proteinase in vivo and in vitro" MATRIX SUPPL, vol. 1, 1992, page 314 XP002081055 see the whole document ---	1-9
Y	SHAPIRA ET AL: "protection against endotoxic shock and lipopolysaccharide-induced local inflammation by tetracycline" INFECTION AND IMMUNITY, vol. 64, no. 3, 1996, pages 825-828, XP002081056 see page 827, discussion ---	1-9
Y,P	GREENWALD ET AL: "in vitro sensitivity of the three mammalian collagenases to tetracycline inhibition" BONE, vol. 22, no. 1, January 1998, pages 33-38, XP002081057 see page 33, right-hand column ---	1-9
A	DRENO ET AL: "in vitro modulation of epidermal inflammatory cytokines (IL-1 alpha, IL-6, TNF alpha) by minocycline" J OF INVESTIGATIVE DERMATOLOGY, vol. 101, no. 3, 1993, page 407 XP002081058 see abstract --- -/--	1-9

2

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	INGHAM: "modulation of the proliferative response of murine thymocytes stimulated by IL-1 and enhancement of IL-1 beta secretion from mononuclear phagocytes by tetracyclines" J ANTIMICROB CHEMOTHER, vol. 26, no. 1, 1990, pages 61-70, XP002081059 see abstract ---	1-9
A	NEBLOCK ET AL: "proteolytic processing of IL-1" BIOL BASED IMMUNOMODULATORS THER RHEUM DIS,1986, pages 257-270, XP002081060 see abstract -----	1-9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/ 05670

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 1-9
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int nal Application No
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP 0184389 A	11-06-1986